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Authors
Herber, Candice B
Ingraham, Holly A

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Should We Make More Bone or Not, As Told by Kisspeptin Neurons in the Arcuate Nucleus

Candice B. Herber, PhD[1] Holly A. Ingraham, PhD[1]

[1] Department of Cellular and Molecular Pharmacology, School of Medicine, Mission Bay Campus, University of California San Francisco, San Francisco, California

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Address for correspondence Holly A. Ingraham, PhD, Department of Cellular and Molecular Pharmacology, School of Medicine, Mission Bay Campus, University of California San Francisco, San Francisco, CA 94158 (e-mail: holly.ingraham@ucsf.edu).

Abstract

Since its initial discovery in 2002, the neuropeptide Kisspeptin (Kiss1) has been anointed as the master regulator controlling the onset of puberty in males and females. Over the last several years, multiple groups found that Kiss1 signaling is mediated by the 7TM surface receptor GPCR54. *Kiss1* mRNA is highly enriched in the basal medial and lateral subregions of the arcuate nucleus (ARC) in the medial basal hypothalamus. Thus, Kiss1^{ARC} neurons reside in a unique anatomical location ideal for sensing and responding to circulating steroid hormones as well as nutrients. *Kiss1* expression is highly responsive to fluctuations of the gonadal hormone, estrogen, with nearly 90% of Kiss1^{ARC} neurons expressing the nuclear hormone estrogen receptor alpha (ERa). Here we review recent research that extends the function of Kiss1ARC neurons beyond the regulation of puberty and highlight their emerging, novel roles in controlling energy allocation, behavioral outputs, and sex-dependent bone remodeling in females. Indeed, some of these previously unknown functions for Kiss1 neurons are quite striking as exemplified by the remarkable increase in bone mass after manipulating estrogen signaling in Kiss1^{ARC} neurons. Taken together, we suggest that Kiss1^{ARC} neurons are highly sensitive to nutritional and hormonal cues that dictate energy utilization and reproduction.

Keywords
- kisspeptin
- estrogen
- arcuate nucleus
- bone

Kisspeptin peptide ligand and its cognate receptor, GPR54 (Kiss1R), together function as a master gatekeeper in mammals to dictate the timing of the onset of puberty. Since its initial discovery as a tumor suppressor in 1996, Kiss1 metastasis suppressor (a.k.a. metastin) is now largely recognized for its role in fertility and reproductive biology. Indeed, a survey of past literature reveals that major research efforts have focused nearly exclusively on kisspeptin-expressing hypothalamic neurons and their control of the initiation of puberty in rodents and humans and in both sexes.[1–8] Recently, however, it has been suggested that central kisspeptin signaling plays a broader role by coordinating the onset of puberty with metabolic parameters, such as nutrient sensing.[9–13] Here, we will discuss an additional and unexpected emerging role of Kiss1^{ARC} neurons in controlling whole-body skeletal homeostasis, highlighting recent studies that link both central and peripheral kisspeptin signaling to bone density and outlining important questions to be answered in this developing field (►**Fig. 1**).

In the brain, kisspeptin is found in both male and female neurons, where it is largely restricted to two hypothalamic clusters, the arcuate nucleus (ARC) and the anteroventral periventricular nucleus (AVPV). The majority of Kiss^{ARC} neurons also express two other peptides, Neurokinin B and dynorphin; hence, these are often referred to as KNDy neurons. Elegant work using chemogenetic and optogenetic approaches demonstrated that a subset of Kiss1^{ARC} neurons function as metabolic sensors that dial up or down energetic outputs during puberty, pregnancy, and lactation. Connections with the adjacent nutrient-sensing agouti-regulated peptide (AgRP) neurons are proposed to integrate metabolic cues with reproductive needs to ensure survival of both the mother and offspring. Surprisingly, this group also reported that only a small number of Kiss1 afferent projections make

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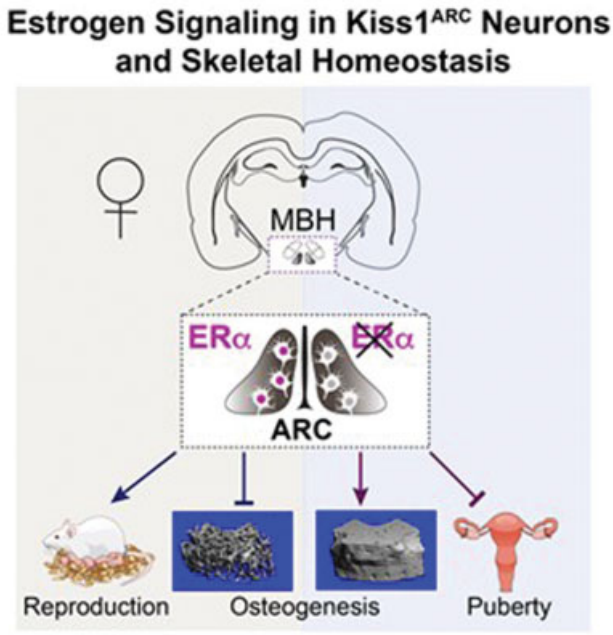

Fig. 1 Abstract showing established role of ERα estrogen signaling in Kiss1^{ARC} neurons in reproduction. Our recent study demonstrates that these same neurons must normally restrain skeletal homeostasis in females. Lifting this restraint by genetic or stereotaxic ablation of ERα in the ARC within the medial basal hypothalamus (MBH) results in high bone density as shown by ex vivo bone micro-CT image of a distal femur isolated from a mutant *Esr1*$^{Kiss1\text{-}Cre}$ female (right) compared with that from a control *Esr1fl/fl* female (left).

functional synaptic connections with gonadotropin-releasing hormone (GnRH) expressing neurons.[14–17] These data suggest that the function of Kiss1^{ARC} neurons is more expansive than simply controlling pulsatile GnRH secretion and the onset of puberty.

Nearly all hypothalamic Kiss1 neurons respond to estrogens, with 99% of Kiss1^{AVPV} and 90% of Kiss^{ARC} neurons thought to express the major nuclear receptor estrogen receptor-α (ERα). There is also a dramatic inverse relationship between the estrogen and Kiss1 pathways as shown by prominent upregulation of *Kiss1* transcripts following deletion of ERα.[18,19] Kiss^{ARC} neurons are proposed to mediate much of the complex negative feedback in the hypothalamic–pituitary–gonadal (HPG) axis by sensing and responding to estrogens as well as nutrient signals including leptin and ghrelin.[20–24] Deleting ERα in the brain, or in a subset of ARC neurons, initially disturbs negative feedback leading to an acute elevation in gonadotropins and estrogen that then normalizes with time.[3,25] This is associated with premature vaginal opening in mice, and the temporary surge in estrogen might contribute to the weak anabolic response in the mouse skeletal system after conditionally deleting ERα in the brain.[26,27] Consistent with this hypothesis, removing all gonadal hormones via ovariectomy in these two models was found to eliminate the weak anabolic response.[26,27]

Kiss1^{ARC} neurons are located in a unique anatomical location ideal for sensing and responding to circulating hormones and nutrients including steroid hormones, fatty acids, insulin, glucose, adiponectin, and calcium.[28,29] Kiss1^{ARC} neurons also express the leptin receptor (LepR), but in far fewer neurons with only 40% expressing both Kiss1 and LepR. Interestingly, intracranial ventricular delivery of leptin in rodents has been reported to have differing effects on bone to either promote or lower bone mass.[26,30–32] Relevant to this discussion, it is unknown whether these bone-induced changes arise from leptin signaling in Kiss1-LepR^{ARC} neurons. As mentioned earlier, orexigenic AgRP neurons in the ARC project to and regulate Kiss1^{ARC} neurons.[33] Stimulation of AgRP neurons by starvation or by optogenetic activation inhibits Kiss1^{ARC} neuron activity and also results in infertility; bone mass was not analyzed in this study. Others have shown that impairing AgRP neuron activity lowers bone mass possibly by increasing sympathetic tone; no link was made to Kiss1^{ARC} neurons.[34] In the developing ARC, nutrient-sensing POMC expressing progenitor cells give rise to all ARC neuronal populations including AgRP and Kiss1 neurons.[35] In this regard, others have found a small increase in trabecular and cortical bone following deletion of ERα in developing POMC neurons (POMC-Cre[26]). Interestingly, in our hands using these same alleles, we failed to detect obvious changes in the female skeleton.[10]

Although no obvious anatomical or functional sex differences within Kiss1^{ARC} neurons have been reported, our laboratory uncovered Kiss1^{ARC} neurons as critical regulators of sex-dependent nutrient partitioning and skeletal metabolism in female rodents using a combination of different genetic models as well as stereotaxic surgery.[10] Deleting ERα signaling in the ARC using the *Esr1* floxed allele[36] and genetic or viral mediated Cre-deletion resulted in a massive increase in female bone mass without changes to food intake. This increase in skeletal density is sex specific, occurring in females only. Female mutants exhibit a stunning increase in trabecular bone mass of approximately 700% with an average 80% BV/TV. Increased bone volume correlates with an elevation in trabecular number and thickness and overall increased mechanical strength of long bones. The high bone mass phenotype is observed in young peripubertal females (4.5 weeks of age) and in older female mice of 1.5 years—an age that is roughly equivalent to a 65-year-old woman. Surprisingly, no changes in circulating estrogen were observed in our genetic models and importantly, the high bone mass persisted in female mice even after ovariectomy (OVX). Additionally, acute deletion of ERα in the ARC in female mice after OVX resulted in a 50% increase in skeletal density, albeit with lower peak bone mass than when circulating estrogen is onboard. Collectively, these data imply that there is a sex-dependent brain-to-bone pathway which remains partially intact even in the absence of ovarian hormones.

This remarkable high bone mass phenotype exceeds that of other reported mouse models including the sclerostin knockout mouse which has approximately 60% volumetric bone mass observed in long bones.[37] Within mutant female bones, one observes an elevation of *Osterix (Sp7)* and Runt-related transcription factor 2 (*Runx2*) levels, suggesting that the increased bone formation in mutant females results from an expansion of pre-osteoblasts. These molecular data

support the observed increase in bone formation rate in the absence of any observed changes in bone resorption markers and histology. An obvious change in sympathetic tone can also be excluded in this model, as catecholamine levels remain unchanged and show normal diurnal patterns. Thus, the very high bone mass in our three different mouse models likely stems from an expansion of pre-osteoblasts rather than an increase in sympathetic tone or decrease in bone resorption.[10] No changes in circulating pituitary hormones were observed, including follicle-stimulating hormone (FSH), a proposed modulator of skeletal homeostasis.[38,39] Nor did we detect changes in circulating gonadal steroids including estradiol or testosterone. Collectively, these negative findings challenge the long-held view that central estrogen signaling in Kiss1^{ARC} neurons is critical for controlling negative feedback on the HPG. Rather, our findings establish a paradoxical role of central estrogen signaling in Kiss1^{ARC} neurons as a major player in female skeletal homeostasis. When engaged, this pathway normally restrains rather than promotes new bone formation. In the future, it would be of interest to know whether direct chemogenetic manipulation of Kiss1^{ARC} neurons, or whether pure or mixed ERα antagonists acting on these neurons, will similarly alter bone mass.

The interplay between estrogen and Kisspeptin is quite dynamic. Interestingly, we and others show that *Kiss1* transcripts or a knock-in GFP reporter are upregulated after deleting ERα.[10,25] This interplay appears to be highly relevant in Kiss1^{ARC} neurons prior to the onset of puberty when stimulation of truncal and axial bone growth is initiated.[40–42] Perhaps part of the initial prepubertal skeletal growth spurt in females just prior to puberty, and with the concomitant rise in gonadal hormones, can be partly attributed to enhanced kisspeptin signaling in the ARC. A more granular approach is needed to define the precise Kiss1 neuronal subpopulation in the ARC that drives the high bone mass phenotype.

Kisspeptin itself appears to act on the bone to induce osteogenesis. Recently, it was shown that Kp-10, the smallest of the Kisspeptins, is able to bind Kiss1R on the surface of C3HT10T1/2 mesenchymal stem cells (MSCs) triggering NFAT4c (nuclear factor of activated T cells 4), a known regulator of osteoblast proliferation and osteoclast precursor recruitment.[43,44] NFATc4 activates bone morphogenic protein (BMP2) which is a key factor in activating osteogenesis by regulating the expression of early osteogenic genes *Runx2* and alkaline phosphatase (*Alp*). Gene editing in these immortalized cells via CRISPR/Cas9 established that the Kp-10-Kiss1R signaling is required for activation of this osteogenic response. Mice deleted for kisspeptin or Kiss1R exhibit approximately 50% decrease in volumetric trabecular bone mass.[45] There are multiple explanations that can account for this phenotype including higher FSH levels, which have been postulated to negatively influence bone mass,[38,39] lower circulating estrogen in mice that are hypogonadal, or impaired osteoblast proliferation in MSCs.

While our data show a strong sex bias with the high bone mass phenotype restricted to females, it is possible that males have a parallel system in other hormone-sensitive brain regions. Finally, identifying the underlying molecular mechanisms responsible for this powerful sex-dependent neuroskeletal pathway has immense discovery potential for new cellular therapeutic targets in age-related bone loss.

Conflict of Interest
None declared.

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